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Adaptor Protein 3BP2 and Cherubism

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Abstract

The adaptor protein 3BP2 (c-Abl Src homology 3 domain-binding protein-2, also referred to SH3BP2) is known to play a regulatory role in signaling from immunoreceptors. In mast cells, 3BP2 is rapidly tyrosine phosphorylated by the aggregation of the high affinity IgE receptor and the overexpression of its SH2 domain results in the dramatic suppression of IgE-mediated tyrosine phosphorylation of PLC-γ, Ca^{2+} mobilization and degranulation. 3BP2 is a substrate of the protein-tyrosine kinase Syk, which phosphorylates it on Tyr^{174}, Tyr^{183}, and Tyr^{446} (in the mouse protein). Phosphorylation of Tyr^{183} promotes the activation of Rac1 through the interaction with the SH2 domain of Vav1. Phosphorylation of Tyr^{446} induces the binding to the SH2 domain of the upstream protein-tyrosine kinase Lyn and enhances its kinase activity. Thus, 3BP2 has a positive regulatory role in IgE-mediated mast cell activation. In lymphocytes, engagement of T cell or B cell receptors triggers tyrosine phosphorylation of 3BP2. Suppression of the 3BP2 expression by siRNA results in the inhibition of T cell or B cell receptor-mediated activation of NFAT. Genetic analyses reveal that 3BP2 is required for the proliferation of B cells and B cell receptor signaling. Point mutations of the *3BP2* gene cause the rare human inherited disorder cherubism, characterized by excessive bone resorption in the jaw bones. These mutations include substitution and deletion mutations of 3BP2. "Cherubism" mice exhibit increased myeloid cell responses to M-CSF and RANKL leading to the activation of osteoclasts. Further analysis could demonstrate that inhibition of 3BP2 might have therapeutic potential.

Introduction

Molecular scaffold, also called adaptor proteins, have indispensable roles to link the receptor-activating signals to downstream effectors by assembling, targeting and regulating signaling molecules. Several adaptor proteins are the substrates of proximal protein-tyrosine kinases (PTKs). These molecules lack catalytic activity, but they have multiple motifs and domains that allow binding to other signaling molecules and therefore act as positive or negative regulators controlling intracellular signal transduction.

In 1993, an adaptor protein 3BP2 and the related molecule 3BP1were isolated through their capacity to bind to the Src homology 3 (SH3) domain of the proto-oncogene product c-Abl in a cDNA expression library screen [1]. Short Pro-rich sequences of 3BP1 and 3BP2 have been shown to mediate association with the c-Abl-SH3 domain. *In vitro* binding experiments demonstrate that Pro-rich regions of 3BP2 preferentially binds to the SH3 domains of c-Abl, c-Src and GRB2, and poorly to those of Nck and neural Src, suggesting that there is specificity in the binding of 3BP2 Pro-rich regions for the different SH3 domains. 3BP1 and 3BP2 are not homologous molecules. 3BP1 contains GAP (GTPase activating proteins) homology domain in the central portion, whereas 3BP2 possesses an SH2 domain at its carboxy terminal region [2,3] (Fig. 1). In 1998, 3BP2 was also isolated as one of the Syk-kinase interacting proteins by yeast two-hybrid screening [4].

Cherubism is an autosomal dominant disease characterized by multiple symmetrical cysts in the mandible and maxilla, excessive bone degradation, and typical facial swelling. Genetic analyses identify several substitution and a deletion mutation in the *3BP2* gene on chromosome 4p16.3 (Table 1) [5-11]. Deletion of 4p16.3 also correlates with Wolf-Hirschhorn syndrome and bladder cancer [12,13]. Here we review the structure and function of 3BP2, implications of 3BP2 mutations in the pathogenesis of cherubism, and the possible role of 3BP2 in intracellular signals of osteoimmune cells.

I. Structure of 3BP2

The 3BP2 protein is composed of a PH (pleckstrin homology) domain, Pro-rich regions, a cherubism region, and an SH2 domain (Fig. 1). The PH domain is required for the interaction with phosphatidylinositol 3,4,5-triphosphate which recruits 3BP2 to the plasma membrane for interactions with other signaling molecules. Thus deletion of the PH domain causes the dysfunction of 3BP2 in T and B lymphocytes [4,14].

The Pro-rich regions (PR1, PR2, and PR3) of 3BP2 are known to associate with the SH3 domain of c-Abl, Src family PTKs (Fyn, Lyn, and Lck), Vav family of guanine nucleotide exchanging factors, CIN85 (Cbl interacting protein of 85 kDa, also referred as CD2AP/SH3KBP1), and HIP-55 (hematopoietic progenitor kinase 1 interacting protein of 55 kDa, also referred as SH3P7/mAbp1) [1,4,14-17]. Domain-deletion studies demonstrate that PR1 is critical for the interaction with Vav1, Vav2, and CIN85 [14,17]. The binding region of CIN85-SH3 domains on 3BP2 is mapped to the Pro-Val-Pro-Thr-

Pro-Arg motif in PR1. On the other hand, PR3 is required for 3BP2 interaction with HIP-55 [17]. PR2 is not involved in the interaction with those molecules.

The SH2 domain of 3BP2 interacts with Syk family PTKs, Syk and ZAP-70 [4]. This interaction of 3BP2 with Syk requires the SH2 domain of 3BP2 and the kinase activity of Syk, suggesting that 3BP2-SH2 domain associates with autophosphorylated Tyr residue(s) in the linker or kinase domain of Syk [4]. The SH2 domain has also been shown to interact with various signaling molecules in different cells. *In vitro* binding studies demonstrate that TCR (T cell receptor) stimulation results in the binding of 3BP2 by its SH2 domain to ZAP-70, LAT, Grb2, PLC (phospholipase C)-γ1, and c-Cbl [4].

In 1994, the specific motifs recognized by the SH2 domain of 3BP2 was analyzed by using a degenerate peptide library [18]. The SH2 domain of 3BP2 has a strong preference for Glu at +1 and Asn at +2 positions after pTyr. Therefore, 3BP2-SH2 domain is thought to bind to the Tyr-Glu-Asn motif. An adaptor protein LAT has two copies of this motif (Tyr^{120} and Tyr^{226}). In fact, 3BP2-SH2 domain associates with LAT after the stimulation of the high affinity IgE receptor (FcεRI) in mast cells [19]. In NK cells, CD244 (also named 2B4) also has the similar sequence (Tyr-Glu-Val) in the cytoplasmic tail within the ITSM (immunoreceptor tyrosine-based switch motif). CD244 is a member of the SLAM (signaling lymphocyte activation molecules) related receptors that are expressed on NK and cytotoxic T cells [20]. Tyr^{337} in this sequence is phosphorylated by cross-linking of CD244, and this phosphorylation of Tyr^{337} allows binding to the 3BP2-SH2 domain [21]. B cell CD19 possesses four copies of the Tyr-

Glu-Asn motif and two of them (Tyr^{403} and Tyr^{443}) interact with the SH2 domain of 3BP2 [22]. Furthermore, the protein-tyrosine phosphatase SHP-1 in pervanadate-treated 293T cells binds to the 3BP2-SH2 domain following phosphorylation of Tyr^{566} in SHP-1 by Lck [23].

3BP2 is tyrosine phosphorylated when it is coexpressed with Syk and Src family PTKs [15,19,24]. Syk phosphorylates 3BP2 on Tyr^{174}, Tyr^{183}, and Tyr^{446} (in the mouse protein) (Fig. 2) [15]. Since Tyr^{183} of 3BP2 is part of the consensus binding sequence for the SH2 domain of Vav1 (Tyr-Leu-Glu-Pro), phosphorylation of Tyr^{183} of 3BP2 may create an interaction site for Vav1-SH2 domain. *In vitro* binding experiments show that a synthesized peptide encompassing phospho-Tyr^{183} of 3BP2 binds to Vav-1, PLC-γ1 and PLC-γ2 from pervanadate-stimulated human NK cells [24]. However, the biological relevance of these bindings remain unclear because treatment of cells with pervanadate, a non-specific inhibitor of protein-tyrosine phosphatase, strongly increases tyrosine phosphorylation of cellular proteins. In mast cells, a point mutation of Tyr^{183} to Phe abrogates the association of 3BP2 with Vav1-SH2 domain (Fig. 2) [25].

Tyr^{174} and Tyr^{446} which are located in the Asp-Tyr-Glu motif, match predicted Syk-substrate sequences as determined by phage display experiments (Fig. 2) [26]. Phosphorylation of Tyr^{446} creates the binding site for the SH2 domain of Lyn in mast cells and Lck in T cells (Fig. 2) [15,16]. Although 3BP2 is a substrate of Syk, phosphorylation of Tyr^{446} in 3BP2 increases phosphorylation of Syk by the activation of

the upstream Src family kinase Lyn, suggesting that 3BP2 is an activator of Syk (Fig. 2) [15]. The role of phosphorylation of Tyr^{174} in 3BP2 is still unclear.

Ser^{225} and Ser^{277} in 3BP2 are the putative phosphorylation sites by cAMP-dependent protein kinase, protein kinase C, or Akt [27]. Phosphorylation of 3BP2 by serine kinases causes the interaction with the scaffold protein 14-3-3; a point mutation of Ser^{277} abrogates this interaction with 14-3-3 and elevates 3BP2-mediated NFAT (nuclear factor of activated T cells) activity in T cells and B cells. In mast cells, expression of 3BP2 with a point mutation of Ser^{277} increases IgE-mediated degranulation (Fig. 2) [25]. These findings suggest that Ser^{277} plays a negative regulatory role in 3BP2-mediated cell function.

Genetic studies of patients with cherubism have identified mutation in 3BP2 including eleven substitutions and one deletion (Table 1). All of these substitution mutations cause change of amino acids located within a limited sequence of 3BP2 (hot spot region of cherubism: Arg^{415}-Ser-Pro-Pro-Asp-Gly^{420}) (in the human protein) (Fig. 1). Within this sequence, substitutions on Ser^{416} and Pro^{417} have not yet been reported. Recently an aggressive case of cherubism was reported with a deletion mutation in 3BP2 (Table 1) [11]. This mutation corresponds to a deletion of cytosine that causes a silent mutation in Arg^{49} (CGC to CG/T) and leads to frame-shift mutations (50 to 74 a.a.) followed by a stop codon (TGA) within the PH domain of 3BP2, with the loss of the Pro-rich regions and the SH2 domain. Thus, this deletion mutation causes the loss of 3BP2 expression.

Histological feature of giant cell granuloma of the jaw is similar to that of cherubism, but 3BP2 is normal. Detection of 3BP2 mutations can be useful in the deferential diagnosis of these conditions [28,29].

II. Physiological Function of 3BP2

In human tissues, 3BP2 mRNA has been detected in spleen, peripheral blood leukocytes, and to a lesser extent in the thymus [4] while PCR screening additionally detects mRNA in the testis and ovary [4]. Moreover, 3BP2 transcripts are observed in primary osteoblasts and cultured osteoclasts [5]. Gene chip analysis shows that *3BP2* mRNA is expressed in bone, oocytes, lungs, and lymph nodes in normal mouse tissues, with especially high expression in B lymphocytes [22]. In cultured cell lines 3BP2 protein is expressed in hematopoietic lineage cells, epithelial cells, fibroblasts, and glioma cells (Table 2). In preliminary experiments we observed 3BP2 expression in the mouse osteoblastic MC3T3-E1 cell line and the mouse macrophage RAW264.7 cells, progenitors of osteoclasts (Hatani and Sada, unpublished observations).

T cells

Engagement of the TCR induces the sequential activation of the nonreceptor type PTKs Lck and ZAP-70 which amplify the receptor initiated signals. Deckert *et al*. was the first to show the adaptive function of 3BP2 in T cells, which had been described previously as a c-Abl SH3-interacting protein of unknown function [4]. In these cells, the overexpression of 3BP2 induces the transcriptional activation of NFAT, AP-1, and IL-2 (interleukin-2) promoter [4]. In T cells, receptor stimulation results in 3BP2

phosphorylation on both serine and tyrosine residues [16,23]; phosphorylation of Ser^{277} is required for the interaction with scaffold protein 14-3-3, and this interaction negatively regulates the 3BP2-mediated NFAT activation [27]. Phosphorylation of Tyr^{183} and Tyr^{446} (in the mouse protein), as well as the SH2 domain of 3BP2 contributes to TCR-mediated activation of NFAT [16]. The decreased expression of 3BP2 with siRNA (short interfering RNA) blocks TCR-mediated activation of the IL-2 promoter and NFAT [16]. These findings demonstrate that 3BP2 has a regulatory function in T cell signaling.

Some 3BP2 translocates to the lipid raft (glycolipid-enriched microdomains: GEMs) in the plasma membrane after receptor stimulation [16,17] with 3BP2 co-localization with CIN85 and HIP-55 at the immunological synapse, T cell-APC interface [17]. Interaction of 3BP2 with these molecules suggests a role for 3BP2 in endocytosis and cytoskeletal rearrangements.

Although 3BP2 is a positive regulator of TCR signaling, the possible abnormality of T cell signaling by the point mutation of 3BP2 does not affect the phenotype of the mouse model of cherubism [30].

B cells

Analysis of the tissue distribution of 3BP2 reveals the predominant expression of 3BP2 mRNA in B cells [4]. 3BP2 is tyrosine phosphorylated following BCR (B cell receptor) aggregation [14] and couples proximal PTKs and Vav family proteins to activate Rho family GTPases. Decreased expression of 3BP2 by siRNA blocks BCR-mediated

activation of NFAT suggesting that 3BP2 is required for BCR coupling to NFAT activation [14]. In 3BP2-deficient mice there is normal T cell development, proliferation, cytokine secretion, and signaling in response to TCR ligation [31]. Although there is increased accumulation of pre-B cells in the bone marrow, there are normal numbers of mature B cells in $3BP2^{-/-}$ mice. However, B cell proliferation, cell cycle progression, and signaling in response to BCR ligation are all impaired. Another study observed perturbation in the peritoneal B1 and splenic marginal-zone B cell compartments and diminish thymus-independent type 2 antigen response in $3BP2^{-/-}$ mice [22]. These findings suggest that 3BP2 is critical for BCR, but not for TCR signaling. T cells could still have a homologous molecule which has a redundant function of 3BP2.

NK cells

Cross-linking of FcγR induces tyrosine phosphorylation of 3BP2 in NK cells [24] and overexpression of this protein in NK cells results in the stimulation of FcγR-mediated cytotoxicity. Both the PH and SH2 domains of 3BP2 are required for its tyrosine phosphorylation and 3BP2-mediated NK cell function. Phosphorylation of Tyr^{183} in 3BP2 causes its interaction with Vav and PLC-γ, and is necessary for the role of 3BP2 in cytotoxicity. 3BP2 also interacts with human, but not murine, CD244 in NK cells [21]; CD244 is a member of the CD150 family and possesses ITSMs in the cytoplasmic region. CD244 ligation causes tyrosine phosphorylation of 3BP2 and recruitment of Vav1 to 3BP2. Overexpression of 3BP2 increases CD244-mediated cytotoxicity, but not IFN-γ secretion. These findings demonstrated that 3BP2 plays a regulatory role on NK cell-mediated cytotoxicity.

Mast cells

Aggregation of the FcεRI induces mast cell activation leading to the release of histamine and production of cytokines. In rat basophilic leukemia RBL-2H3 cells, FcεRI aggregation results in rapid 3BP2 tyrosine phosphorylation (Fig. 2) [19]. Phosphorylation of Tyr^{446} contributes to the activation of Lyn suggesting that 3BP2 might be one of the factors regulating Lyn in FcεRI signaling (Fig. 2) [15]. Phosphorylation of Tyr^{183} creates the binding sites for Vav1-SH2 domain which regulates the activation of Rac1 (Fig. 2) [25]. Overexpression of mutant forms of 3BP2 provides insights to the function of endogenous molecule. The FcεRI-mediated activation of IKK, JNK, ERK, NFAT, and cytokine production are all attenuated by the overexpression of the cherubism 3BP2 mutants. Overexpression of 3BP2-SH2 domain results in a suppression of FcεRI-mediated tyrosine phosphorylation of PLC-γ, Ca^{2+} mobilization and degranulation (Fig. 2) [19]. Expression of 3BP2 with a point mutation of Ser^{277} to Ala results in the enhancement of degranulation, cytokine production, and cellular signaling (Fig. 2) [25]. Thus, phosphorylation of Ser^{277} may be involved in the negative regulation of mast cell function through the interaction with 14-3-3. Altogether these observations demonstrate that 3BP2 regulates IgE-mediated cytokine production and degranulation.

III. Implications of 3BP2 mutations in the pathogenesis of cherubism

The studies summarized in the previous sections indicated the physiological function of 3BP2 in lymphocytes and mast cells. To understand the pathological role of 3BP2 in cherubism, Ueki *et al* introduced the most common mutation found in cherubism patients,

Pro^{416} to Arg (Pro^{418} to Arg in humans) into the mouse *3BP2* gene [30]. The homozygote of these "cherubism" mice ($3BP2^{KI/KI}$) exhibits trabecular bone loss, enhanced osteoclast differentiation in tissues, TNF-α-dependent systemic inflammation, and cortical bone erosion. Mutant myeloid cells show increased responses to M-CSF (macrophage-colony-stimulating factor) and RANKL (RANK (receptor activation of NF-κB) ligand) stimulation, and differentiate to macrophages expressing high levels of TNF-α and unusually large osteoclasts.

The systemic inflammation observed in cherubism mice is not improved by crossing these mice with RAG1-deficient mice (homozygous for the *3BP2* mutant allele and null for *Rag1*) which lack T and B cells, but the phenotype is rescued by crossing with TNF-α-null mice [30]. Therefore although 3BP2 is a positive regulator of TCR and BCR signaling, mutation of 3BP2 in lymphocytes, T and B cells, was not the basis of the phenotype of cherubism mice. Moreover, the absence of TNF-α rescues trabecular bone loss, but not enhanced osteoclastogenesis in response to M-CSF and RANKL in cherubism mice [30]. This indicates that in cherubism mice TNF-α-mediated bone loss and stimulated osteoclastogenesis are regulated by different mechanisms in macrophages and osteoclasts.

Expression of the mutant form of 3BP2 causes sustained ERK phosphorylation in macrophages and increased Syk Tyr^{346} phosphorylation in osteoclasts. Phosphorylation of Tyr^{346} in Syk (in the mouse protein) is elevated in $3BP2^{KI/KI}$ mutant osteoclasts [30]. Overexpression of the wild type and cherubism mutant form of 3BP2 increases M-CSF

and RANKL-mediated phosphorylation of Tyr^{346} in Syk, whereas phosphorylation of Tyr^{317} or Tyr^{519}/Tyr^{520} (in the mouse protein) is not affected by the cherubism mutation of 3BP2. The mechanism by which the linker region Tyr residues (Tyr^{317}, Tyr^{342}, and Tyr^{346}) in Syk are phosphorylated in the process of osteoclastogenesis remains unclear. Perhaps a member of the Src family PTKs or Syk itself phosphorylates Tyr^{346} [30]. The kinase activity of Syk is not affected by the cherubism mutation of 3BP2 because phosphorylation of Tyr^{519}/Tyr^{520} in the activation loop of the kinase domain, which correlates with the kinase activity, is normal. Altogether the observations of Ueki *et al* demonstrate that cherubism is a TNF-α dependent hematopoietic disorder [30].

VI. Perspectives: Roles of 3BP2 in osteoimmune signals

Cherubism results from the imbalance between the function of osteoblasts and osteoclasts in the jaw bone, both of which expresses 3BP2 [5]. The protein-tyrosine kinase Syk, several immunoreceptor tyrosine-based activation motif (ITAM)-bearing receptors or αvβ3 integrin have essential roles in the development of osteoclasts and activation of NFATc1 [32-34]. In addition, one of the Vav family proteins, Vav3, has a critical role in the regulation of osteoclasts [35]. Since 3BP2 is a substrate of Syk and regulates the activation of Vav family proteins in immune cells, it is likely that 3BP2 plays an important role in osteoimmune signals in osteoclasts (Fig. 3). We previously demonstrated that overexpression of 3BP2 has a potential to affect the activation of IKK, JNK, and ERK [25]. Therefore, it is possible that dysfunction of 3BP2 affects the activation of those kinases in osteoclasts (Fig. 3).

Abl-deficient mice are osteoporotic and have defects in osteoblast maturation suggesting that Abl has an essential role in the development of osteoblasts, cells which also express 3BP2 [5,36]. Since 3BP2 is a binding molecule of c-Abl, the cherubism mutation of 3BP2 could affect the normal development and function of osteoblasts.

Because cherubism is a rare disease affecting only a limited number of patients, it is unclear whether these patients have immunological defects. A recent report of an aggressive case who died of septicemia suggests an immunocompromised state [11]. However, this case was due to a deletion mutation that resulted in the lack of the expression of full-length 3BP2. Although in vitro 3BP2 is a positive regulator of lymphocytes, the phenotype in cherubism mice was not affected by the lack of T and B cells [30]. Thus, the phenotype in cherubism mice is not dependent on 3BP2 function in lymphocytes. Therefore, further analysis with selective inhibition of 3BP2 might have therapeutic potential.

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Footnotes

The abbreviations used are: PTKs, protein-tyrosine kinases; SH, Src homology; PH, pleckstrin homology; CIN85, Cbl interacting protein of 85 kDa; HIP-55 (hematopoietic progenitor kinase 1 interacting protein of 55 kDa; TCR, T cell receptor; LAT; linker for activation of T cells; PLC, phospholipase C; FcεRI, high affinity IgE receptor; ITSM, immunoreceptor tyrosine-based switch motif; NFAT, nuclear factor of activated T cells; IL-2, interleukin-2; siRNA, short interfering RNA; BCR, B cell receptor; M-CSF, macrophage-colony-stimulating factor; RANKL, RANK (receptor activation of NF-κB) ligand; ITAM, immunoreceptor tyrosine-based activation motif; CN, calcineurin.

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Table 1

Mutations of 3BP2 in cherubism

Wild type	Mutant	References
Substitutions		
Arg^{415} (CGA)	Pro (C**C**A)	[5]
	Gln (C**A**A)	[5]
Pro^{418} (CCC)	Leu (C**T**C)	[5]
	Arg (C**G**C)	[5,7,30]
	His (C**A**C)	[5]
	Thr (**A**CC)	[10]
Asp^{419} (GAT)	Asn (**A**AT)	[8]
	Gly (G**G**T)	[9]
Gly^{420} (GGG)	Glu (G**A**G)	[5]
	Arg (**C**GG)	[5]
	Arg (**A**GG)	[6]
Deletion		
Arg^{49} (CGC)	Arg (CG/**T**)	[11]

Table 2

Cultured cell lines expressing 3BP2

Cells			References
T cells	Human	Jurkat	[4,16,24]
		CEM	[4]
		HUT-78	[4]
		KT-8	[24]
	Mouse	DBHy3	[4]
		B3Z	[4]
B cells	Human	Raji	[4,14,24]
		C1R	[24]
		CRL	[24]
		BJAB	[14]
		RPMI8866	[14]
		Daudi	[14]
NK cells	Human	YT	[21]
Mast cells	Rat	RBL-2H3	[19,25]
Monocytes	Human	U937	[4,24]
Myeloid cells	Human	K562	[24]
Epithelial cells	Human	HeLa	[24]
Fibroblasts	Human	GM847	[24]
Glioma cells	Human	Mo59K	[24]

Figure Legends

Fig. 1 Structure of 3BP2.

3BP2 is composed of a PH domain, Pro-rich regions (PR1-3), and an SH2 domain. Both human and mouse a.a. numbers are shown. Positions of point mutations are shown at the bottom.

Fig. 2 Role of 3BP2 in mast cells.

3BP2 plays regulatory roles in IgE-mediated mast cell activation.

Fig. 3 Possible role of 3BP2 in osteoimmune signaling. Dysfunction of 3BP2 in cherubism may affect the signaling pathways leading to the activation of NFATc1 in osteoclasts. M-CSFR: M-CSF receptor. Ig-like receptor: immunoglobulin-like receptor.

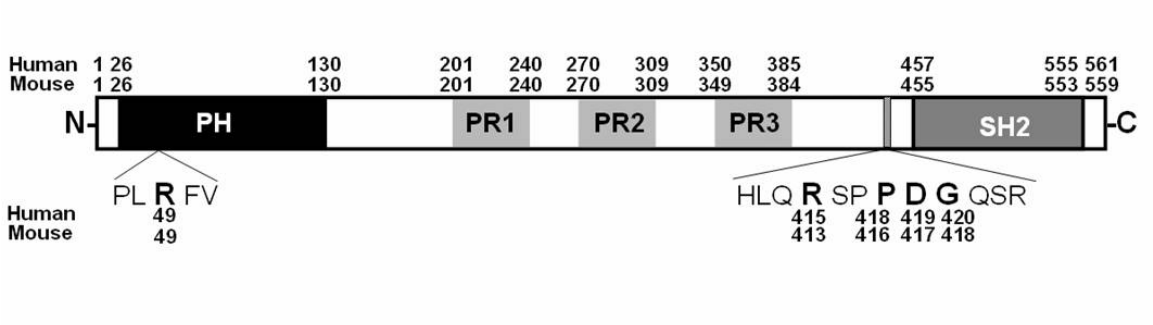

Fig. 1. Hatani and Sada

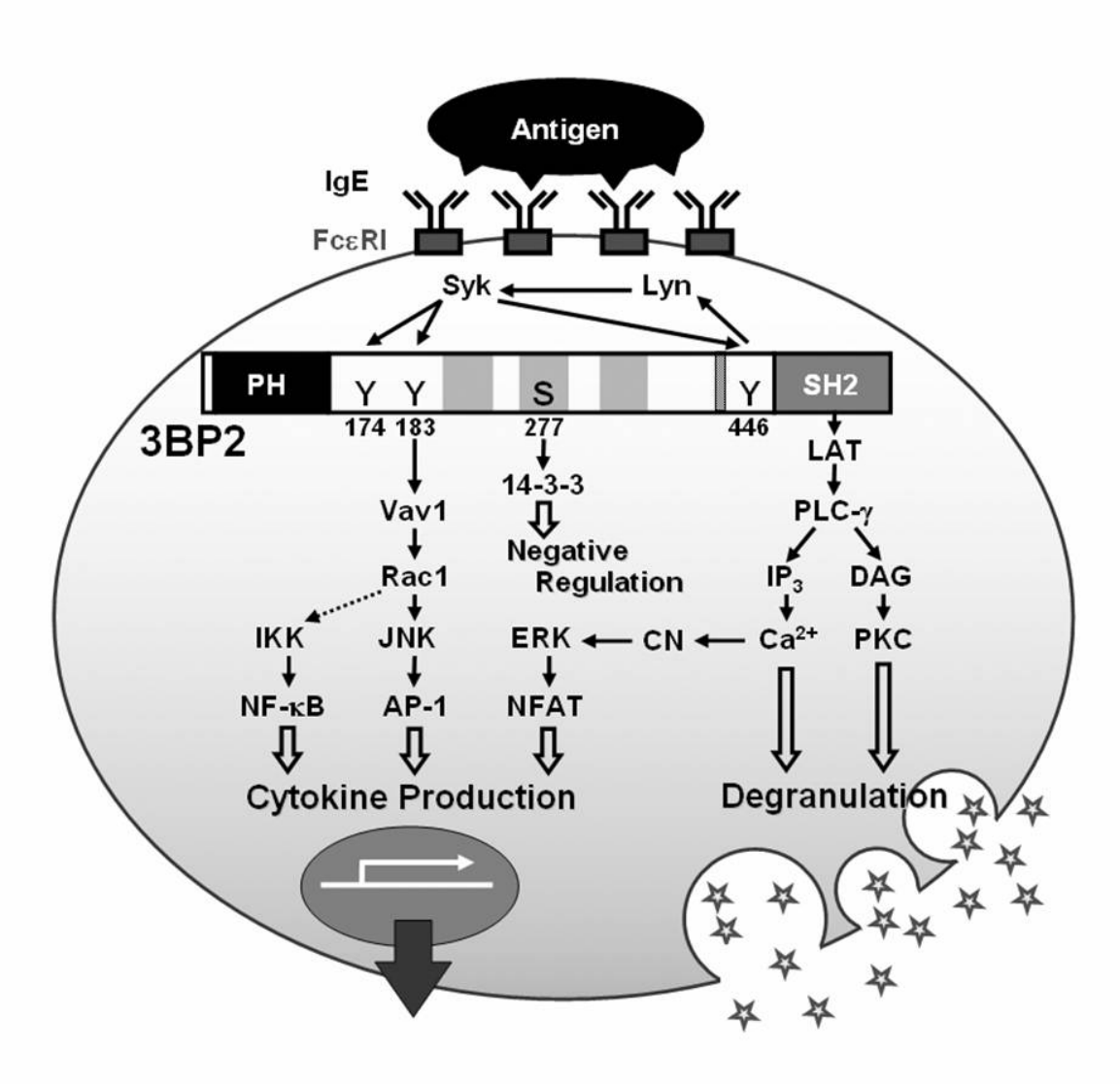

Fig. 2. Hatani and Sada

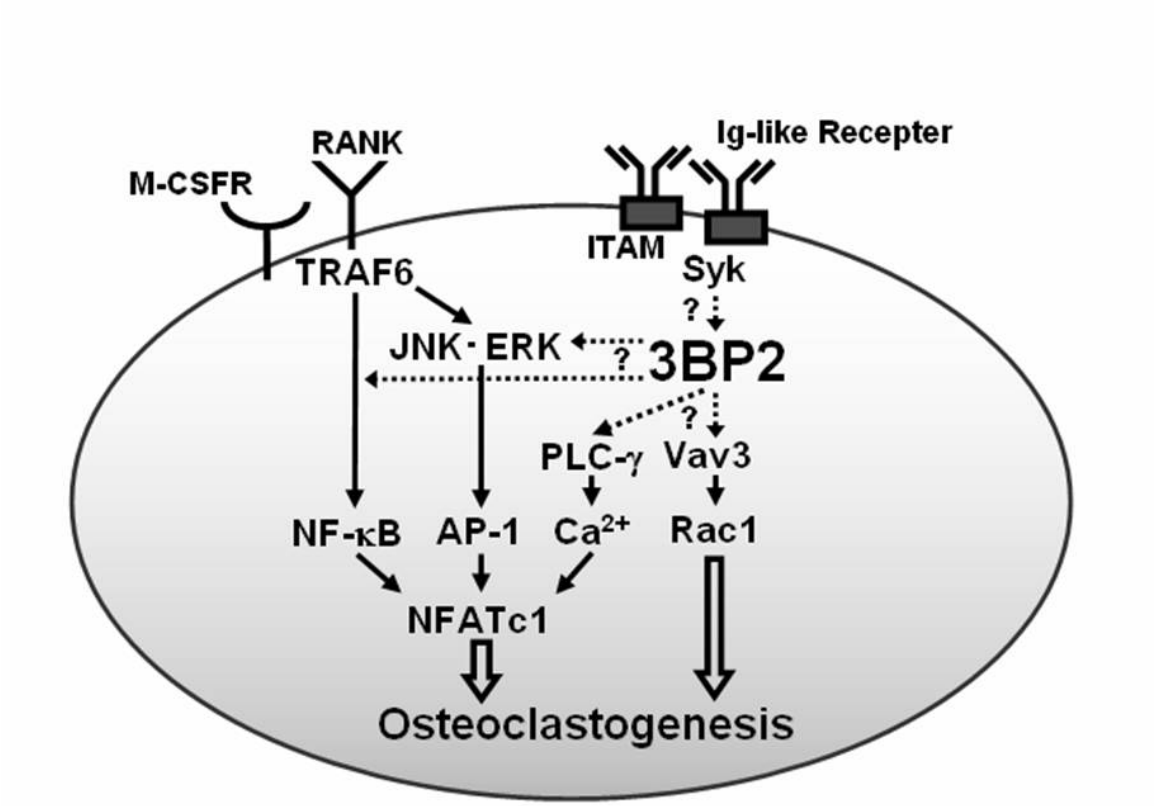

Fig. 3. Hatani and Sada